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13. ABSTRACT *(Maximum 200 Words)*

Organic compounds were shown to become sequestered in soil with a consequent decrease in biodegradability, availability for animal uptake, and toxicity. The rate and extent of sequestration varied among soils with dissimilar properties. Sequestration could be increased or decreased by appropriate soil treatments. Sequestration and bioremediation caused a marked diminution in availability of organic compounds to animals. Because of sequestration, current methods for analysis give inappropriate estimates of exposure and risk from persistent pollutants. Analytical methods to assess changes in bioavailability resulting from sequestration were developed. Among the compounds evaluated were several polycyclic aromatic hydrocarbons, triazines, and chlorinated hydrocarbons. Data were obtained in support of hypotheses suggesting that nanopores or partitioning into organic matter is responsible for sequestration.

14. SUBJECT TERMS

Sequestration, toxicity, soil, biodegradation, bioavailability/bioremediation

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OBJECTIVES

1. Measure the rate and extent of sequestration/aging of organic compounds that results in their reduced availability for microbial degradation in soils of different properties.

2. Determine whether a solvent-extraction method can predict the extent of reduced bioavailability of organic compounds for biodegradation in soil.

3. Establish the effect of soil type on the rate and extent of reduction in bioavailability to individual animal, plant and microbial species as organic compounds age (become sequestered) in soil.

4. Determine whether the reduction in biodegradability or extractability with selective solvents as a result of sequestration is a predictor of the diminished uptake or toxicity to higher organisms as organic compounds age in soil.

TECHNICAL SUMMARY

Phenanthrene and atrazine were aged for 200 days in sterilized samples of 16 soils that differed greatly in physical and chemical properties. At regular intervals during the aging period, the extent of sequestration was determined by measurement of biodegradation of the compounds by bacteria and the amounts extracted by a mild extraction procedure. Both compounds became sequestered in each of the soils, but the rate and extent of sequestration varied markedly among the soils. Sequestration was largely complete in some soils in 120 days, but it extended for longer periods in others. The extent of sequestration of the two compounds in the 16 soils was not highly correlated. The declines in bioavailability in the soils were not highly correlated with the decreases in extractability or

the amounts of unextracted compounds, although soils in which the declines in bioavailability were greatest showed the greatest declines in extractability. Because of the marked differences among soils, we conclude that generalizations about the rate and extent of sequestration in soils are not yet possible.

We further analyzed the same 16 soils to determine their porosity in the range of 7 nm-10 μm, cation-exchange capacity, surface area, and clay mineralogy. Based on these and previously determined properties of the soils, correlations were sought between soil characteristics and 4 methods of measuring sequestration. Simple correlation analysis showed that some but not all measures of phenanthrene and atrazine sequestration were correlated with organic C content or nanoporosity but not other properties of the soils. Multiple linear-regression analysis suggested an interaction of organic C content with clay content/cation-exchange capacity and with nanoporosity/surface area in determining the extent of atrazine sequestration. Therefore, certain properties of soil may be useful predictors of sequestration of some compounds, but it is not yet possible to use those properties to predict sequestration of other compounds.

A study also was conducted to determine the relationship between organic matter content of soil and the availability of aged phenanthrene. Phenanthrene was aged for 200 days in sterile samples of dissimilar soils, soils treated with H_2O_2 to reduce the content of organic matter, and sand. Sequestration as measured by the extent of mineralization of phenanthrene by an added bacterium was appreciable in samples with >2.0% organic C, and the bioavailability of the hydrocarbon declined with time of aging. Sequestration was not evident in soils or sand with <2.0% organic C. More of the compound remained after biodegradation of the hydrocarbon aged for 200 days than if it was not aged, with the largest amount remaining in soils with >2.0% organic C and the least in sand. Aging as measured by a decline in extractability of 1-butanol was evident in all soils, although the rate

was fastest in soil with >2.0% organic C. The volume occupied by pores of <10-μm diameter was higher in soils containing more organic matter and was negligible in sand. These data indicate that the organic matter content of soil is a major determinant of sequestration.

We conducted an investigation of factors affecting the sequestration and changes in bioavailability as phenanthrene persists in soils. Phenanthrene became sequestered in soils differing appreciably in organic matter and clay content as measured by earthworm uptake, bacterial mineralization, or extractability. Phenanthrene also became sequestered as it aged in soil aggregates of various sizes as measured by decline in availability to a bacterium, a mild extractant, or both. Wetting and drying a soil during aging reduced the amount of phenanthrene recovered by a mild extractant and the rate and extent of bacterial mineralization of the hydrocarbon. After biodegradation of phenanthrene added to the soil, more of the compound remained if it had been aged than if it had not been aged. Wetting and drying the soil during aging further increased the amount of phenanthrene remaining after biodegradation. The rate and extent of bacterial mineralization of phenanthrene were less in leached than in unleached soil. Aging/sequestration is thus markedly affected by environmental factors.

The effect of wetting-drying cycles was investigated further. Subjecting soil to wetting-and-drying cycles during periods of aging <60 days decreased the biodegradability, extractability, and uptake by earthworms of phenanthrene and reduced the extractability of di(2-ethylhexyl) phthalate (DEHP) sequestered in soil as compared to soil aged at constant moisture. The mineralization of sequestered DEHP was greater in soil that was wet and dried during a 41-day period of aging than in soil incubated at constant moisture. Wetting and drying soil during periods of aging of 100 or more days had no effect on the biodegradability or assimilation by *Eisenia foetida* of sequestered phenanthrene and DEHP.

Subjecting soil containing previously sequestered phenanthrene to 1, 3, and 4-wetting-and-drying cycles increased the biodegradability of the compound. The extractability of sequestered phenanthrene was greater in soil that was wet and dried once after aging than in soil maintained at constant moisture, but 3 wetting-and-drying cycles did not affect extractability. The biodegradability of sequestered DEHP was unaffected by wetting and drying. These observations indicate that wetting and drying may be useful in the remediation of contaminated soils.

We then further explored ways to increase the biodegradability of compounds that have aged in soil or aquifer material and become less bioavailable. Slurrying enhanced the rate and extent of biodegradation by individual bacterial strains of aged and unaged phenanthrene and DEHP in soils and aquifer solids. After bacterial degradation of aged phenanthrene in unslurried soil had largely ceased, the residual compound was metabolized if the soil was slurried and reinoculated with a phenanthrene-degrading bacterium. The rate and extent of biodegradation of aged phenanthrene by *Pseudomonas* sp. were enhanced when anthracene or pyrene was added to the soil at the same time as the bacterium, although the organism could not metabolize anthracene or pyrene. Moreover, anthracene or pyrene increased the amount of aged phenanthrene removed from soil by a mild extractant. The data show that the bioavailability of organic compounds that become sequestered by aging can be altered by appropriate soil treatment.

We also carried out an investigation to determine the combined effect of sequestration and laboratory-scale bioremediation on the bioavailability of polycyclic aromatic hydrocarbons in soil. After the compounds had aged for 140-203 days in soil, bacteria capable of degrading the compounds were added, and the availability of the hydrocarbons after bioremediation was determined. Aging decreased the amount of phenanthrene, anthracene, fluoranthene, and pyrene available to bacteria as shown by

increases in the amount of the compounds remaining after bioremediation and to earthworms (*Eisenia foetida*) as shown by lower tissue concentrations, percentages assimilated, and bioconcentration factors. Aging also diminished the availability of anthracene to wheat and barley. Bioremediation caused a marked diminution in the amount of phenanthrene, fluoranthene, and pyrene taken up by earthworms. The smallest amounts of these three compounds were assimilated from soil in which they had aged and then been subject to biodegradation. The results show that the combined effects of sequestration and bioremediation lead to a more marked reduction in bioavailability than either process alone.

In further evaluations of the effect of sequestration on other species, house fly (*Musca domestica*), fruit fly (*Drosophila melanogaster*), and German cockroach (*Blattella germanica*) were used to assay the acute toxicity of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and dieldrin aged in sterile soil. Significant reduction in morality of the insects was observed in DDT- and dieldrin-amended soil samples aged for 30 days, and toxicity decreased further with additional aging. After 270 days, DDT and dieldrin were no longer toxic to house flies, and dieldrin was no longer toxic to fruit flies and cockroaches at 120 days. Nevertheless, 84.7% of the DDT and 92.1% of the dieldrin was recovered from the soil by vigorous extraction after 180 and 270 days, respectively. The results show that toxic chemicals residing in soil become less toxic with time, the extent of decline in bioavailability may differ among different species, and vigorous extraction may grossly overestimate toxicity.

In assessments of bioavailability to other species, earthworms (*Eisenia foetida*) were found to assimilate decreasing amounts of atrazine, phenanthrene, and naphthalene that had been incubated for increasing periods of time in sterile soil. The amount of atrazine and phenanthrene removed from soil by mild extractants also decreased with time. The declines

in bioavailability of the three compounds to earthworms and of naphthalene to bacteria were not reflected by analysis involving vigorous methods of solvent extraction. It is thus evident that regulations based on vigorous extractions for the analyses of persistent organic pollutants in soil do not appropriately estimate exposure or risk to susceptible populations.

For purposes of measuring the effect of sequestration on carcinogens, we developed a genotoxicity assay for samples from environments in which toxic organic compounds are largely sorbed. The assay entails measurement of the rate of mutation of a strain of *Pseudomonas putida* to rifampicin resistance. The ratio of induced to spontaneous mutants was a function of the concentration of a test mutagen in soil. In studies of the utility of the assay in samples amended with 2-aminofluorene as a test mutagen, the ratio of induced to spontaneous mutants declined with time. The decline paralleled the disappearance of extractable 2-aminofluorene from the soil. The ratio of induced to spontaneous mutants also fell in four other soils with dissimilar properties. This solid-phase assay appears to be more appropriate for the estimation of genotoxicants sorbed in soil than assays involving extractants or suspensions of soil or sediment samples.

We also examined the feasibility of devising a chemical assay to predict the bioavailability of organic compounds that become sequestered in soil. The recovery of atrazine and phenanthrene freshly added to soil varied appreciably among individual solvents, but the quantity extracted by each solvent declined as the test compounds persisted in soil. The percentage recovered by some extractants approximated either the percentage uptake by earthworms or bacterial degradation. Recovery by one extractant predicted bioavailability to both organisms. The data suggest that it is feasible to predict bioavailability of persistent organic compounds in soil by chemical procedures.

For evaluation of possible mechanisms of sequestration, a study was conducted using model solids to determine whether the time-dependent decline in availability for biodegradation of organic pollutants in soil might result from the entrapment of these compounds in porous or nonporous solids. A strain of *Pseudomonas* mineralized phenanthrene in solid alkanes containing 18 to 32 carbons, three waxes, and low-molecular-weight polycaprolactone, polyethylene, and polypropylene. The rates were appreciably slower than when the substrate was not initially present within these nonporous solids. From 1.4 to 63.4% of the polycyclic aromatic hydrocarbon added to the solids was mineralized in 90 days. The rates and extents of partitioning of phenanthrene varied markedly among the solids. The rates of partitioning and biodegradation of phenanthrene initially present in the alkanes were positively correlated. The bacterium rapidly and extensively mineralized phenanthrene provided in calcium alginate beads containing varying amounts of soluble soil organic matter. The rates and extents of phenanthrene mineralization declined as the percentage of the substrate in the nanopores within silica particles increased, but the reductions in rate, extent, or both were less pronounced than with nonporous solids. The rate of 4-nitrophenol biodegradation also declined with increasing percentages of the compound in these nanopores. The data are consistent with hypotheses that the sequestration and consequent decrease in bioavailability of organic compounds that persist in soil result from their partitioning into organic matter or their presence within nanopores in soil.

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